Interactive effects of composted green waste and earthworm activity on tree growth and reclaimed soil quality: a mesocosm experiment.

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Abstract

On reclaimed landfill sites, the addition of organic matter such as composted green waste (CGW) to soil-forming materials can support tree survival and growth. CGW addition may also assist the establishment of sustainable earthworm populations, and in turn these organisms can promote further soil development through their burrowing and feeding activity. Despite such potentially mutual benefits, little research has been carried out into CGW and earthworm interactions with trees on reclaimed land. A twelve month, open field nursery experiment revealed the responses of the interactions between two tree species; *Alnus cordata* (Betulaceae) and *Acer platanoides* (Sapindaceae), CGW and the earthworms *Aporrectodea longa* (Lumbricidae) and *Allolobophora chlorotica* (Lumbricidae) in reclaimed soil. Controlled mesocosm conditions permitted a detailed investigation into the factors affecting tree growth and nutrient uptake, soil nutrient cycling and earthworm population dynamics. Results revealed that *A. cordata* growth was unaffected by CGW or earthworm addition. There was, however, a significant positive synergistic effect of earthworm activity and CGW addition on *A. platanoides* growth. CGW addition significantly increased levels of organic carbon and essential plant macro-nutrients in reclaimed soil while earthworm activity assisted decomposition of both leaf litter and CGW. Findings showed that CGW may serve as a suitable early source of organic matter to support earthworm population establishment on reclaimed sites. This experiment demonstrates that CGW improves reclaimed soil quality, thereafter supporting tree establishment and growth on reclaimed landfill.

Keywords: Landfill restoration; tree species; organic waste; soil quality; synergistic effect

Introduction

Creation of a suitable soil resource is essential for sustainable greenspace establishment, to provide necessary soil chemical and physical conditions and restore normal soil biological functions (Scullion, 1992). There is increasing industrial and scientific interest in improving the soil materials used in reclamation projects, particularly through the addition of organic matter from waste streams, such as Composted Green Waste (CGW) (Foot *et al.*, 2003; Moffat, 2006; Nason *et al.*, 2007; Forest Research, 2015). However, at present there is limited research into the effect of CGW on tree growth and soil quality on reclaimed land (Ashwood *et al.*, 2014). The few available field experiments have demonstrated some benefits of CGW on tree establishment, and this appears to be dependent on the rate and depth of incorporation (Foot *et al.*, 2003; Moffat *et al.*, 2008).

The addition of organic waste materials to reclaimed soil may also enable the establishment of sustainable earthworm populations, which can in-turn support tree growth and the delivery of ecosystem services (Lowe and Butt, 2002, 2004; Blouin *et al.*, 2013). Certain earthworm species (e.g. anecic, deep-burrowing) actively incorporate and mix organic waste materials into soils, enhancing mineralisation and benefiting soil fertility (Piearce and Boone, 1998; Lowe and Butt, 2002). The addition of earthworms may therefore be an effective way to enhance the benefits of organic wastes such as CGW during land reclamation. However, studies into the utilisation of earthworms during the restoration of brownfield sites to woodland are few in number, and have experienced limited success, particularly due to inappropriate earthworm species selection and the use of excessively hostile substrates without sufficient amendment (see the reviews of Butt, 1999, 2008). Those studies which have investigated the influence of earthworms on forest tree species in natural soils have mostly observed a positive influence of earthworms on tree growth (e.g. Marshall, 1971; Haimi *et al.*, 1992; Muys *et al.*, 2003; Welke and Parkinson, 2003; Larson *et al.*, 2010). However, such results are unlikely to be directly comparable to the specific conditions presented by reclaimed land.

There is overwhelming agreement that tree species differently influence soil quality and soil faunal population development through the quality and quantity of their leaf and root litter (Swift *et al.*, 1979; Pigott, 1989; Muys *et al.*, 1992; Reich *et al.*, 2005; Rajapaksha *et al.*, 2013). It is therefore of value, when planning land reclamation to a woodland end-use, to understand whether the tree species planted are likely to provide litter which enables native soil faunal communities to establish, thus supporting soil development and local ecosystem service provision (Kibblewhite et al., 2008; Rajapaksha et al., 2013). Certain tree species, such as *Alnus cordata* and *Acer platanoides* are recommended for planting on reclaimed or ex-industrial land, based on their tolerance to high soil pH and dry soil conditions, and potential for SRF based on fast growth rates (Hibberd, 1986; Forest Research, 2011). Currently however, there is a paucity of knowledge regarding the interaction between these two non-native tree species and native UK soil biota, making these pertinent tree species to investigate further and compare to previous research with similar native species (Rajapaksha *et al.*, 2013; Ashwood *et al.*, 2017).

The aim of this mesocosm experiment was to investigate the interaction effects between earthworms, trees and soil quality after CGW addition, in order to inform future land restoration activities. The experimental design is based upon the study done by Rajapaksha *et al.* (2014), which successfully demonstrated a beneficial earthworm-tree interaction between native UK earthworms and an exotic eucalypt tree species. Specific objectives of the present study were to: (i) measure the effect of composted green waste (CGW) and earthworm activity on tree growth and nutrient uptake in reclaimed soil; (ii) investigate the effects of CGW and tree species on earthworm community density in reclaimed soil; (iii) assess the effects of CGW, tree species, earthworm activity and their interactions, on reclaimed soil carbon and nutrient status.

2. Materials and Methods

2.1. Study site and experimental design

The experiment was located at the Forest Research Headley Nursery Enclosure, Hampshire (Nat. Grid Ref: TQ 54929 84214), previously used for similar experiments (McKay *et al.*, 1999; Moffat, 2000; Broadmeadow *et al.*, 2005; Rajapaksha *et al.*, 2014). It utilised a planting-tube mesocosm technique, similar to that employed by Rajapaksha *et al.* (2014). The mesocosms consisted of 0.25 m diameter, 3 mm thick PVC tubes cut to 0.6 m lengths. The base of each tube was covered with fine mesh (1 mm, supplied by Amari Plastics) to prevent earthworm ingress/egress. Earthworms were further confined inside the open-top mesocosms through the application of two unbroken strips of adhesive plastic hook ('velcro') tape applied to the inside of the tubes, following the design of Lubbers and van Groenigen (2013). Tubes were buried in the ground to 0.4 m depth, with 0.2 m protruding above ground level. This technique allows removal of whole soil/root system from the tube at termination of the experiment and permits detailed examination for desired soil depths and has been successfully used for tree root experiments (Bending and Moffat, 1997) and tree growth/earthworm interaction experiments (Rajapaksha *et al.*, 2014). Each tube was filled to 0.4 m depth with a soil treatment, and a tree was planted in the middle of each tube (detail in 2.2.). The experiment began in June 2014 and ran until July 2015.

Figure 1 shows the layout of tubes within the experimental plot, which consisted of five blocks, each containing a randomised placement of 9 planting tubes (4 treatments X 2 tree species, and 1 soil-only control). Each block contained a representative of each tree species in all four treatment combinations, and a tree-free control tube, which contained de-faunated reclaimed soil only, to account for the effect of tree species alone on soil parameters. Each of the nine tree-treatment combinations had five replicates, totalling 45 tubes in this experiment. Each block was separated by a 3-m buffer zone, and within the blocks, each planting tube was separated by 1.5 m. The wider experimental plot itself was homogenous and each planting tube was separated from the surrounding soil. As such, each tube acted as an individual experimental unit (e.g. replicate), irrelevant of location on-site. The perimeter of the experiment location was surrounded by an electrified rabbit-proof fence to prevent damage to trees by small herbivorous mammals. Following tree planting, a continuous drip irrigation system was applied to each tube to maintain soil moisture level at 25-30% for optimal tree growth (Figure 2).

[INSERT FIGURE 1]

A single Prenart Super Quartz soil water sampler (PTFE suction cup lysimeters, 25 mm diameter, 95 mm length) was installed in each tube, within the upper 0.1 m of the soil profile, to allow for soil solution samples to be taken by connecting it to a vacuumed bottle. These were subsequently found to be unable to remove sufficient soil water samples for chemical analysis, despite soil in tubes being kept sufficiently moist through irrigation (perhaps due to high clay and stone fraction of reclaimed soil media preventing good contact) and so this method of sampling was abandoned.

[INSERT FIGURE 2]

2.2. Experimental treatments

This experiment employed four treatment combinations: no treatment (control); CGW addition only; mixed-species earthworm addition only; and CGW and mixed-species earthworm addition. For CGW-treated tubes, the soil included incorporation of screened 0-25mm PAS 100 "Soil Improver" grade CGW (courtesy of Viridor Ltd) at a rate equivalent to 500 kg Total N ha^{-1}, which amounted to 31.4 g $tube^{-1}$. This amendment rate was chosen to reflect the legal limit set by Nitrates Directive for Nitrate Vulnerable Zones (NVZs), following guidance of Taylor (1991) and Bending *et al*. (1999). A full summary of CGW nutrient content is provided in Table 1.

[INSERT TABLE 1]

Fresh reclaimed soil was collected from Ingrebourne Hill Community Woodland, a 54-ha area of reclaimed land in Rainham, Essex, UK (Nat. Grid Ref: TQ 52572 83192). The soils at Ingrebourne Hill comprise generally of sandy clay loam materials, with a high stone content (Heaven and Richardson, 2007). Metal contents were within the UK soil guideline values for non-residential uses, and not considered to be at levels harmful to fauna (Doick and Willoughby, 2011). Further soil data are provided in Table 2. Following removal from the site, soil was initially de-faunated in bulk by placement into 30 l sealed plastic containers, and stored at -5°C for 7 days to destroy native earthworms and other potential competitors/predators (Butt, 2011). It was then allowed to thaw before being fully homogenised using a cement-mixer, which was cleaned thoroughly before use, and some disposable soil initially run though to collect any potential contaminants. The homogenised soil was placed into clean tonne soil bags ready for addition to mesocosms. The volume of soil was measured to replicate the 1.06 g cm^{-3} mean bulk density observed at Ingrebourne Hill (Ashwood, 2016) (23.3 kg wet soil was added per tube).

[INSERT TABLE 2]

Soils were left to settle in the mesocosms for one week prior to tree planting. One-year-old root-trainer seedlings (the standard age for trees planted in the field) of Norway maple (*Acer platanoides*) and Italian alder (*Alnus cordata*) were obtained from a local nursery, planted in the mesocosms and left for 2 weeks before earthworm introduction. This experiment investigated two earthworm species: *Aporrectodea longa* and *Allolobophora chlorotica*, which represent ecological groups considered more beneficial to soil development (anecic and endogeic, respectively, sensu Bouché, 1977). These earthworm species have previously shown tolerance for the soil conditions typically presented by reclaimed landfill (Butt and Lowe, 2004; Butt, 2008; Ashwood *et al.*, 2017). All earthworms were collected from pasture at Walton Hall Farm, Preston, UK (Nat. Grid Ref: SD 55050 28100), via digging and hand-sorting of soil, then transferred and stored in fresh soil collected from Ingrebourne Hill, before transporting to Headley Nursery. For mesocosms receiving an earthworm treatment (n=20), introduction was a mixed culture of *A. longa* (n=5) and *A. chlorotica* (n=10). These numbers were based on recorded field densities at an experiment at Ingrebourne Hill, Essex, following inoculation with *A. longa* (Ashwood, 2016), and in keeping with numbers used by Rajapaksha *et al*. (2014). The *A. chlorotica* used in this experiment were of mixed pink and green morphs (Lowe and Butt, 2008), however all were selected to be of similar biomass, and morph was not considered to be a limiting factor as reproductive output was not one of the measurements.

2.3. Experimental sampling

Visual surveying of all tubes was undertaken weekly during autumn, with the number of leaves of both tree species on the soil surface recorded per tube. After 12 months, the mesocosms were carefully dug out of the ground, ensuring that the fine mesh still covered the base and kept the experimental mesocosm unit intact. Mesocosms (containing soils and trees) were transported to an on-site workshop for processing, where each tube was opened using a portable circular saw to allow access to the undisturbed soil column inside (Figure 3). The tree height and ground-line diameter was recorded. The above-ground section of the tree was removed by severing at the ground-line, and apportioned into three sub-samples for analysis; main stem; branches; and leaves. The soil column and plant roots were divided into two sections; shallow (0 to 0.2 m depth) and deep (0.2 to 0.4 m depth). Earthworms were hand-sorted from the soil in each section and numbers recorded. These were preserved in 4% formaldehyde solution for identification in the laboratory, following the key of Sims and Gerard (1999).

[INSERT FIGURE 3]

Both the shallow and deep soil sections were divided into bulk and rhizosphere (root-attached) soil. Rhizosphere soil was obtained by shaking the roots from each section inside a clean plastic sample bag. Live root samples were then divided into the two sub-categories of main root (stump and roots >2 mm diameter), and fine roots (<2 mm diameter) from each soil section. Before chemical analysis, all root samples were jet-washed through a fine sieve (0.5 mm) to remove attached soil. Plant and soil samples were processed at Forest Research Laboratory Services at Alice Holt Lodge, Farnham, UK. A random sample of 100 leaves was taken from each tree to have Specific Leaf Area (SLA) ($cm^2\ g^{-1}$) per dry weight calculated, following European forest monitoring protocols (Pitman *et al*., 2010). This was conducted using a Delta-T Area Meter (MK2) linked via video camera to a Delta-T Conveyor Belt Unit Area Measurement System (Delta-T devices, Cambridge, England). All plant material was then oven-dried at 70°C for 48 h, the dry biomass recorded and samples analysed chemically. Plant and soil samples had total organic C and N determined using a CN Elemental Analyser (Carlo Erba (THERMO), FLASH EA 1112 Series), and major elements (P, K, Ca and Mg) analysed after sulphuric acid digestion and inductively coupled plasma-optical emission spectrophotometry (ICP-OES) analysis, soil moisture content analysed by oven drying at 105°C for 24 hours, and soil pH was measured in 1:2.5 soil/water suspension. The 1M KCL-extraction method was used following the procedure described in MAFF (1986) on fresh soil to provide filtered samples for determining levels of inorganic "available" nitrogen, e.g. NO_3^--N and NH_4^+-N by colorimeter analysis.

2.4. Measurements and statistical analysis

Earthworm density and community change were measured to assess the effects of tree species and soil treatment. The effect of soil treatment and/or earthworm activity on tree growth and health were measured via data on tree survival, tree nutrient status, SLA, ground-line diameter, and above and below-ground tree biomass. Bulk and rhizosphere soil samples were chemically analysed for soil pH, total organic C and N, major elements and soil moisture content to investigate the effect of tree, CGW addition and earthworm activity on soil quality. Data were first tested for normality using the Shapiro-Wilk test, which is suited to the sample size in this experiment (n=5). As all data for each species and treatment had a normal distribution, the data were analysed using one and two-way analysis of variance (ANOVA), with the Tukey-Kramer post-hoc multiple comparison test applied to significant treatment interactions. Analysis of baseline soil data were performed using a 2-sample student's *t*-test. Statistical analysis was performed using the statistical software GenStat (Release 16.2).

3. Results

3.1. The effects of earthworm activity and CGW addition on tree survival, growth, biomass and nutrient status

Both tree species achieved 100% survival across all treatments. *A. cordata* clearly demonstrated greater growth than *A. platanoides* throughout the experiment, across all treatments. At the start of the experiment there was no significant difference in individual height or diameter of trees between treatments, for each tree species. At the termination of the experiment, no effect on *A. cordata* height and ground-line diameter was found under the CGW or earthworm treatments, or combination of the two (Figures 4 and 5). *A. platanoides* showed greater height (ANOVA, df = 3, F = 3.60, $p < 0.05$), but not diameter, under the earthworm plus CGW treatment than the control group for this species (Figure 4).

[INSERT FIGURE 4]

[INSERT FIGURE 5]

No effects of treatment were found on the biomass of either tree species. Stem C content (%) of *A. cordata* was increased in both earthworm treatment and earthworm plus CGW treatment (mean ±SE = 50.23 ± 0.12 and 50.24 ± 0.22, respectively) compared with the earthworm-free control group (mean ±SE = 49.36 ± 0.01) (ANOVA, df = 3, F = 8.75, $p < 0.001$) in the upper soil level, fine root Ca levels (%) of *A. cordata* were higher (ANOVA, df= 3, *F* = 3.82, p <0.05) in the earthworm-only treatment (mean ±SE = 1.69 ± 0.06) than the CGW-only treatment (mean ±SE = 1.41 ± 0.08). The average C: N ratios for *A. cordata* and *A. platanoides* leaves was 19.4 and 37.5, respectively. No treatment effect on the Specific Leaf Area (SLA) measurements of either tree species was found.

3.2. The effects of tree and soil treatments on earthworm populations

At termination of the experiment after 12 months, there was significantly higher *A. chlorotica* density in mesocosms containing *A. platanoides* with CGW treatment than all other treatments and tree species tubes in the experiment (ANOVA, df = 3, *F* = 5.75, p = 0.002). There were no significant differences in *A. longa* density between tree species or treatments (Table 3). Under *A. cordata*, *A. chlorotica* population density reduced by 78% in both treatments, and by 50% and 82% under *A. platanoides* with and without CGW, respectively. Comparatively, *A. longa* experienced higher survival rates, with an average reduction in final population density of 24% and 8% under *A. cordata* with and without CGW, respectively. Under *A. platanoides*, *A. longa* density was reduced by 36% and 32% under CGW and earthworm only treatment, respectively.

[INSERT TABLE 3]

At termination of the experiment, some of the earthworm-free control tubes were found to contain low numbers of *A. longa* and *A. chlorotica*. The mean (±SE) density of *A. longa* per control tube was 0.5 (± 0.3) and 0.7 (± 0.3) for *A. cordata*, and *A. platanoides,* respectively. *A. chlorotica* mean density (±SE) per control tube was 0.6 (± 0.4) and 0.2 (± 0.1) for *A. cordata* and *A. platanoides*, respectively. No other earthworm species were found in the earthworm-control tubes in the experiment, and no earthworms were recovered in the tree-free control tubes. A total of 3 individuals of *L. rubellus* were found within two tubes (containing CGW plus earthworm treatment) during sampling, representing a mean density (±SE) of 0.03 (± 0.07) per tube.

3.3. Soil responses to earthworm activity, CGW addition and tree species presence

At the start of the experiment, there was little variability (CV<10%) in soil chemical parameters between the sub-samples of CGW-free soil (Table 4, and Table V in supplementary material). CGW addition led to increased soil pH (p<0.05), and higher total N, organic C, organic matter content and PO_4^{3-} (p <0.001). Addition of CGW was associated with a reduction in soil Ca content (p <0.05), C:N ratio, NO_3^- and SO_4^{2-} (p <0.001) compared with baseline soils.

[INSERT TABLE 4]

At termination of the experiment, soil chemistry results for bulk soil under trees in the CGW-free and earthworm-free controls showed several effects of tree species, compared with the tree-free control soils (Table 5, and Table VI in supplementary material). Under *A. cordata*, there was higher (p <0.05) soil organic C (%) and organic matter (%), and lower (p <0.001) soil moisture content (%), and plant nutrients K, Mg, and PO_4^{3-} (mg/kg) (p<0.05). Bulk soil under *A. platanoides* was not different to the control soil, except in moisture content (%), which was lower than the control (ANOVA, df = 1, *F* = 7.43, p <0.001), but also higher (ANOVA, df = 1, *F* = 8.70, p <0.001) than under *A. cordata*.

[INSERT TABLE 5]

Underneath both tree species, several effects were observed on bulk and rhizosphere soil quality due to CGW and earthworm addition. In all cases, deeper soil sections (0.2-0.4 m) did not provide sufficient rhizosphere (root-attached soil) for performance of chemical analysis. *A. cordata* bulk soil in the shallow 0.2 m soil section had a higher (p <0.05) soil organic carbon and organic matter content in the CGW-earthworm combination treatment than in the tree-free control shallow soil section (Tables I and VII, supplementary material). Total N was higher (p <0.001) in soils receiving treatments containing CGW than control soil. Bulk soil C:N ratio was higher in the earthworm only treatment ($p < 0.05$) than the CGW-only treatment. Soil moisture content (%) and K and NO_3^- content was higher ($p < 0.001$) in the tree-free control, compared with the control and the earthworm-only treatments under *A. cordata*. In the 0.2 m rhizosphere soil, the only differences were in the CGW-only treatment, which had the lowest C:N ratio (ANOVA, df = 3, $F = 8.31$, $p < 0.001$) and highest content of PO_4^{3-} (ANOVA, df = 3, $F = 3.45$, $p < 0.05$). In the 0.2-0.4 m deep soil under *A. cordata*, organic C, organic matter and total N were higher ($p < 0.001$) under CGW-only and earthworm plus CGW combination treatments.

A. platanoides bulk soil in the shallow 0.2 m soil section had a higher total N, soil organic carbon and organic matter content in both the CGW-only and CGW-earthworm combination treatments than the controls and earthworm-only treatments ($p < 0.001$). Under the CGW-only treatment, levels of K, NH_4^+ and PO_4^{3-} were higher ($p < 0.05$) than control and earthworm-only tubes (Tables II and VIII, supplementary material). Bulk soil, in both the shallow and deep sections, had higher C:N ratio in earthworm-only treatment than the tubes receiving CGW treatments. In the 0.2-0.4 m deep soil under *A. platanoides*, soil total N, organic matter and PO_4^{3-} were higher under the and earthworm plus CGW combination treatments ($p < 0.05$).

4. Discussion

4.1. The effects of CGW and earthworm activity on tree growth and nutrient uptake in reclaimed soil

After 12 months in the mesocosms, *A. cordata* outperformed *A. platanoides* in growth, across all treatments. In a comparable field experiment, Foot *et al.* (2003) found that under a similar application rate of CGW to capped landfill sites, *A. cordata* significantly outperformed sycamore (*A. pseudoplatanus*). This was attributed to greater availability of N to the nitrogen-fixing alder species, through its association with *Frankia* bacterium, and the *A. cordata* in the experiment presented here also showed development of root nodules. In the current study, a positive synergistic effect of CGW and earthworm activity was observed on *A. platanoides* height, final ground-line diameter and percentage diameter increase. This is in direct contrast to the findings of Ashwood (2016), who found no effect of CGW or earthworm addition on *A. platanoides* growth rates in reclaimed soils in a field experiment, which could be due to drought stress in comparison to soil moisture controlled mesocosms in this study. This is can be explained as an extension of Leibig's Law of the Minimum (Von Liebig, 1840), which states that the fertility of a soil for a particular plant is determined by the availability of the limiting nutrient. In this case, the improved growth response of *A. platanoides* was due to the removal of a limiting factor - soil moisture - rather than addition of a limiting nutrient (Gea-Izquierdo *et al.*, 2009). With adequate soil moisture, this tree species was subsequently able to demonstrate a response to the soil nutrient increases by CGW addition and earthworm activity. The significantly greater density of *A. chlorotica* found under this tree species may also have contributed to such benefits (Edwards, 2004).

No significant effect of CGW or earthworm treatments was found for *A. cordata* height, ground-line diameter or biomass, similar to Foot *et al.* (2003). In field conditions, this species has demonstrated high drought tolerance (Ashwood, 2016) and is not nitrogen-limited; therefore additional nutrient supply may not increase growth of these species as much as for nutrient-limited trees. It is therefore likely that earthworm processing of decomposing organic matter is of less benefit to such hardy and N-fixing trees species.

In our experiment, earthworm presence significantly increased the C content of *A. cordata* stem and Ca levels in fine roots (0 - 0.2 m soil) under the earthworm only treatment compared with the CGW-only treatment. Similarly, Wolters and Stickan (1991) found higher C content in stems of beech (*Fagus sylvatica*) seedlings, when grown in forest soils with an individual *Octolasion lacteum* (an endogeic, geophagous earthworm species), compared with controls. The mechanisms behind earthworm-induced increases in plant growth and nutrient acquisition are generally accepted to be enhanced organic matter decomposition and nutrient mineralisation (Marshall, 1971; Haimi *et al.*, 1992).

4.2. The effects of CGW and tree species on earthworm populations

In this experiment, there was similar density of *A. longa* across all mesocosms, irrespective of treatment or tree species. There was, however, a significantly greater population of *A. cholorotica* under *A. platanoides* with CGW present. In natural systems *A. chlorotica* (an endogeic species) dwells within the rhizosphere and forms close associations with the root systems of plants (Sims and Gerard, 1999). Therefore, root chemistry might be expected to affect this earthworm species through root exudates, or by modifying local soil pH (Dakora and Phillips, 2002; Rajapaksha *et al.*, 2014) although no such effects on soil were observed in our study. *A. longa* population density experienced a mean reduction of 20% during the experiment, and *A. chlorotica* experienced a 50-80% reduction in population density. It is notable that such relatively low earthworm survival rates still enabled clear effects of earthworm activity to be observed. Research indicates that earthworm-mediated improvements in soil nutrient availability, and subsequent benefits to plant growth, are likely to be greater in nutrient-poor soils (Jana *et al.*, 2010). In higher quality soils, plants may be less affected by nutrient limitation and hence any benefits from earthworm activity harder to detect (Brown *et al.*, 2004). Whilst earthworm survival rates were relatively low, the final population densities of earthworms in the mesocosm tubes (a minimum of 64 m^{-2} and 36 m^{-2}, for *A. longa* and *A. chlorotica*, respectively) are not unusual when compared with those commonly found on young reclaimed landfill sites. For example, Pizl (2001) recorded an earthworm density of 66.7 m^{-2} after one year on an afforested colliery spoil in Czech Republic, and Judd and Mason (1995) recorded approximately 80 m^{-2} on a four year old reclaimed landfill site in the UK.

Under laboratory conditions, the generation time of both species (cocoon to mature adult) fits within the twelve month timeframe of the present experiment (Lowe and Butt, 2005), indicating that soil conditions and/or food availability may not have been suited to earthworm reproduction. However, similar mortality rates observed by Rajapaksha *et al.* (2014) in a better quality soil in mesocosms of the same size suggests that earthworm mortality may have been influenced by stress from addition to mesocosms, rather than specific soil conditions. Under field conditions, CGW has been shown to support populations of both *A. chlorotica* and *A. longa* in reclaimed soil for at least 2 years following surface application (Lowe and Butt, 2004). Finding conflicting earthworm survival results between a pot and a field experiment, Butt (1999a) suggested that for some organic wastes, pot experiments may be an unreliable indicator of earthworm performance in natural systems; our results indicate that this may indeed be the case for CGW. He attributed this to issues such as sterilisation of soil and therefore absence of interaction with plants and fauna, and a build-up of ammonia and salts, to which earthworms are sensitive. It is also possible that longer experimental duration may have enabled earthworm populations to recover following seasonal additions of organic matter from leaf litter once CGW has been fully utilised by earthworms, as the foliar material of both tree species has been shown to be palatable to these earthworm species (Ashwood *et al.*, 2017).

4.3. The effects of CGW, tree species, earthworm activity and their interactions, on reclaimed soil carbon and nutrient status

CGW addition affected bulk soil carbon and nutrient levels with significantly higher shallow 0-0.2 m soil organic carbon (%) and organic matter (%) in the CGW-earthworm combination treatment under both tree species, compared to the CGW-only treatment. This may be explained by leaf litter accumulation in the soil through earthworm activity. Bohlen and Edwards (1995) found that earthworms increased the amounts of extractable N and NH_4^+ from manure and legume organic waste treatments. However, in our study, under both tree species the levels of soil total N and NH_4^+ were significantly higher in the CGW-only treatment. Interestingly, this suggests that earthworm activity did not increase the level of extractable NH_4^+ released from CGW beyond typical CGW decomposition rates, or this was consumed by the earthworms. Since soil pH was high, these may also have been rapidly converted to NO_3^- form or NH_4^+ bound to clay lattices in the soil. Earthworm activity was associated with significantly higher C:N ratio in the shallow section bulk soil under both tree species, and the deep bulk soil under *A. platanoides*, compared with earthworm-free controls. This is likely due to earthworm activity distributing organic matter from surface leaf litter into the deep soil level (Lowe and Butt, 2003) and high utilisation of available N. For example, Welke and Parkinson (2003) found that lower horizon mineral soil contained significantly higher organic matter content in the presence of the endogeic (shallow burrowing) earthworm species *Aporrectodea trapezoides.*

Tree roots of both species increased the levels of C, N, and availability of cations in the rhizosphere compared with bulk soil, and reduced pH and the availability of K^+ and anions. Increases in organic carbon are likely due to root exudation of organic compounds and root turnover (Day *et al.*, 2010; Lukac and Godbold, 2011). Furthermore, trees can influence rhizosphere nutrient supply through biological N fixation (e.g. by alder species), and through the extraction of nutrients, especially nitrate; with subsequent effects on pH and the mobility of other chemicals, e.g. anions and cations (Day *et al.*, 2010).

5. Conclusions

This study provides evidence that CGW can improve tree growth under the limiting conditions found in reclaimed soils, and that earthworm activity can provide a positive synergistic effect on this. However, this may not be the case for tree species which are not limited by nutrient availability or abiotic stress, e.g. alder species. For tree species such as these, simple measures such as irrigation may provide the necessary conditions for their growth and survival. Earthworm population densities showed that CGW addition may serve as a suitable source of organic matter to support earthworms on reclaimed sites. However, a higher rate of CGW application than used in this study is likely to better support earthworm populations and enhance the associated benefits to soil quality and tree growth. Survival rates also indicated that *A. longa* was better suited than *A. chlorotica* to the soil materials used. CGW was shown to significantly improve the reclaimed soil through increased levels of organic carbon and essential plant macro-nutrients.

Future studies would benefit from a longer duration, e.g. minimum of 24 months, to allow tree and earthworm biomass and soil data to reflect any longer-term effects of organic waste application. Additionally, although the CGW application rate used in this study was reflective of legal limits, studies investigating higher application rates of CGW and other organic waste materials and mixtures may identify further opportunities to improve sustainable woodland and earthworm population establishment on reclaimed land.

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Table 1. Viridor 0-25 mm PAS 100 Composted Green Waste summary nutrient analysis (source: technical document supplied by Viridor).

Parameter	% Dry Mass	Kg t^{-1} Fresh Weight	% nutrient availability (in first year)
Nitrogen	1.27	6.20	10
Phosphate	0.19	0.93	75
Potash	0.79	3.86	90
Magnesium	0.26	1.27	60
Sulphur	0.25	1.22	30
Organic Matter	60.20	293.78	n/a

Table 2. Selected soil parameters of Ingrebourne Hill, five years after restoration (data from Doick and Willoughby, 2011).

Parameter (unit)	Value
Extractable-N (mg l^{-1})	0.19
pH	7.9
C:N	29
Organic Matter (%)	4.0

Table 3. Mean (±SE) earthworm density, expressed in in No. mesocosm^{-1} (and equivalent m^{-2}), after 12 months in PVC tubes containing experimental tree species and soil treatments. Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05.

Earthworm species	Baseline density	*A. cordata*		*A. platanoides*	
		Earthworm only (in)	Earthworm and CGW	Earthworm only	Earthworm and CGW
A. longa	5 (100)	4.6 ± 0.25[a] (92 ± 5)	3.8 ± 0.59 [a] (76 ± 11.8)	3.4 ± 0.51 [a] (68 ± 10.2)	3.2 ± 0.59 [a] (64 ± 11.8)
A. chlorotica	10 (200)	2.2 ± 0.73[a] (44 ± 14.6)	2.2 ± 0.66 [a] (44 ± 13.2)	1.8 ± 0.37 [a] (36 ± 7.4)	5.0 ± 0.63 [b*] (100 ± 12.6)

Table 4. Mean (± SE) baseline (t=0) chemical parameters of control soil and compost-amended soil. Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05 **, p <0.01, *** p <0.001.

	Treatment	
Chemical parameter	Control soil	CGW-amended soil
pH	7.93 ± 0.02a	8.02 ± 0.03b*
Cond. (μs/cm)	3043 ± 8	2814 ± 127
Total N (%)	0.11 ± 0.00a	0.14 ± 0.00b***
C (Org) (%)	2.00 ± 0.04a	2.36 ± 0.05b***
O.M. (%)	3.44 ± 0.07a	4.07 ± 0.08b***
C:N ratio	28.04 ± 0.38a	24.44 ± 0.36b***
Moisture content (%)	20.58 ± 1.00	31.10 ± 0.60
K (mg/kg)	4029 ± 151	3876 ± 21
Ca (mg/kg)	33593 ± 743a	30802 ± 718b*
Mg (mg/kg)	3652 ± 61	3647 ± 121
Na (mg/kg)	318 ± 13.40	323.90 ± 11.20
P (mg/kg)	705 ± 64.29	729.78 ± 13.40
S (mg/kg)	1717 ± 57a	1203 ± 121b**
[N(NH_4^+)] (mg/kg)	2.75 ± 0.29	2.92 ± 0.17
[N(NO_3^-)] (mg/kg)	11.46 ± 0.09	2.307 ± 1.24b***
S(SO_4^{2-}) (mg/kg)	1310 ± 54a	584 ± 77b***
P(PO_4^{3-}) (mg/kg)	29.51 ± 0.31a	39.38 ± 0.65b***

Table 5. Effects of tree species on mean (± SE) bulk soil chemical parameters after 12 months. Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05 **, p <0.01, *** p <0.001.

Chemical parameter	Control (no tree)	Tree species	
		A. platanoides	*A. cordata*
pH	9.08 ± 0.44	8.90 ± 0.08	8.78 ± 0.34
Cond. (µs/cm)	828 ± 100	1187 ± 171	1697 ± 335
Total N (%)	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
C (Org) (%)	1.72 ± 0.03a	1.79 ± 0.04a	1.86 ± 0.05b*
O.M. (%)	2.97 ± 0.06a	3.08 ± 0.06a	3.21 ± 0.08b*
C:N ratio	22.88 ± 1.04	23.19 ± 1.13	23.73 ± 1.02
Moisture content (%)	27.14 ± 0.84a	22.55 ± 1.46b***	17.01 ± 1.18c***
K (mg/kg)	123.93 ± 2.66a	111.05 ± 4.36a	87.85 ± 4.08b***
Ca (mg/kg)	2881 ± 314	2940 ± 204	3059 ± 215
Mg (mg/kg)	66.38 ± 4.97a	66.66 ± 5.27a	59.23 ± 4.46b*
Na (mg/kg)	14.85 ± 0.49	15.34 ± 0.83	16.21 ± 0.73
[N(NH_4^+)] (mg/kg)	1.06 ± 0.05	1.03 ± 0.08	0.70 ± 0.12
[N(NO_2)] (mg/kg)	0.36 ± 0.22	0.57 ± 0.35	0.26 ± 0.15
[N(NO_3^-)] (mg/kg)	0.52 ± 0.06a	0.41 ± 0.03a	0.18 ± 0.02b***
S(SO_4^{2-}) (mg/kg)	87.49 ± 13.53	209.08 ± 57.97	287.06 ± 79.67
P(PO_4^{3-}) (mg/kg)	20.38 ± 0.59a	21.11 ± 0.34a	17.36 ± 1.04b***

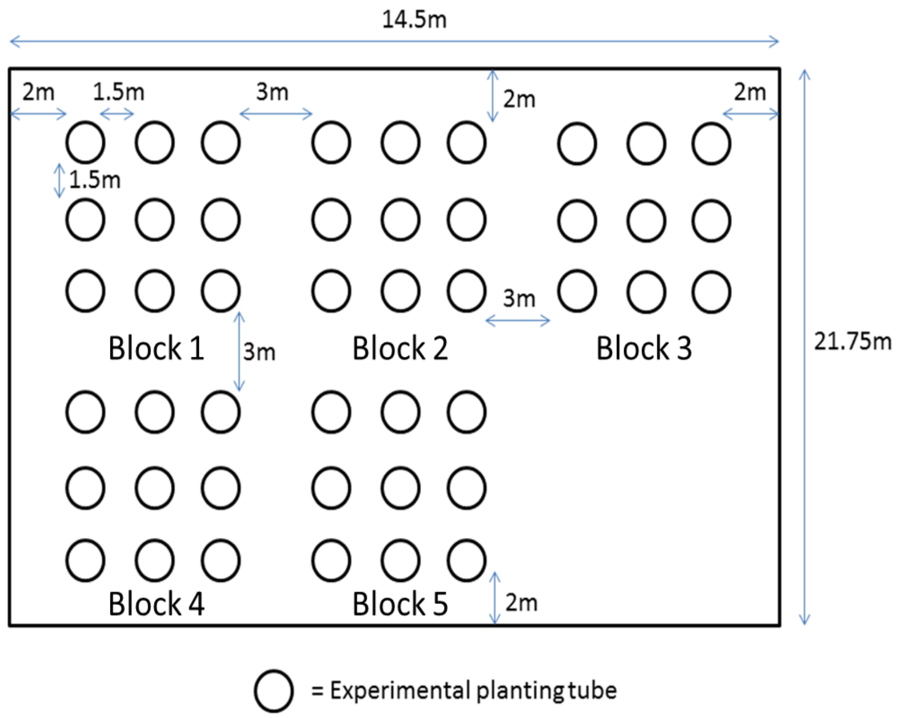

Figure 1. Experimental layout of planting tubes at Headley Nursery.

Figure 2. Inspection of mesocosms. In the foreground the drip-irrigation system is visible, as are the white velcro strips within the experimental tubes to prevent earthworm escape.

Figure 3. Destructive sampling of soil columns at termination of the experiment. A Norway maple (*Acer platanoides*) tube, cut with a circular saw to reveal the soil column and tree root system.

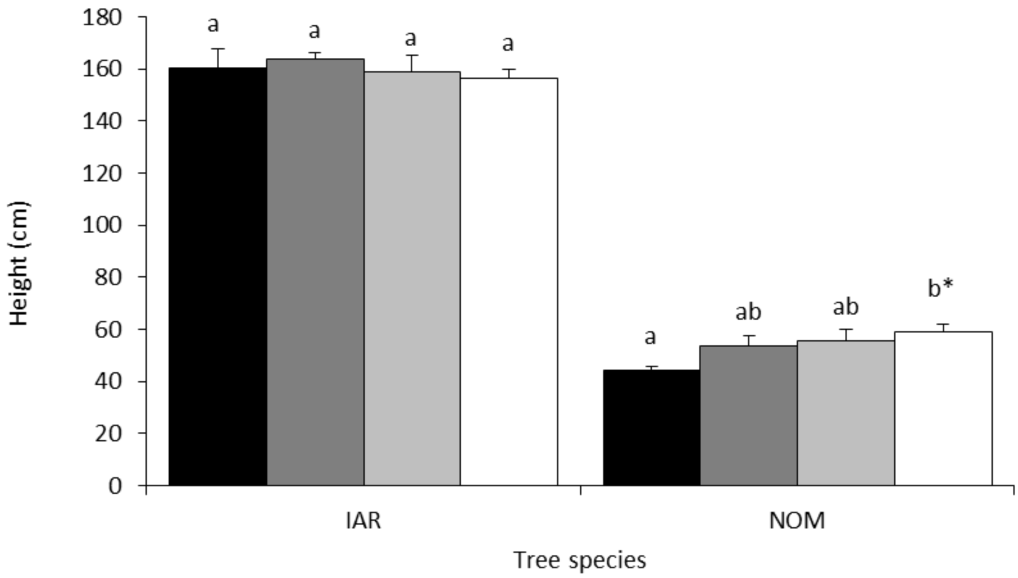

Figure 4. Mean (± SE) height (cm) of *Alnus cordata* (IAR) and *Acer platanoides* (NOM) after 12 months under experimental treatments: Control (■), Earthworm only (■), composted green waste (CGW) only (□), CGW plus earthworm (□). Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * $p < 0.05$.

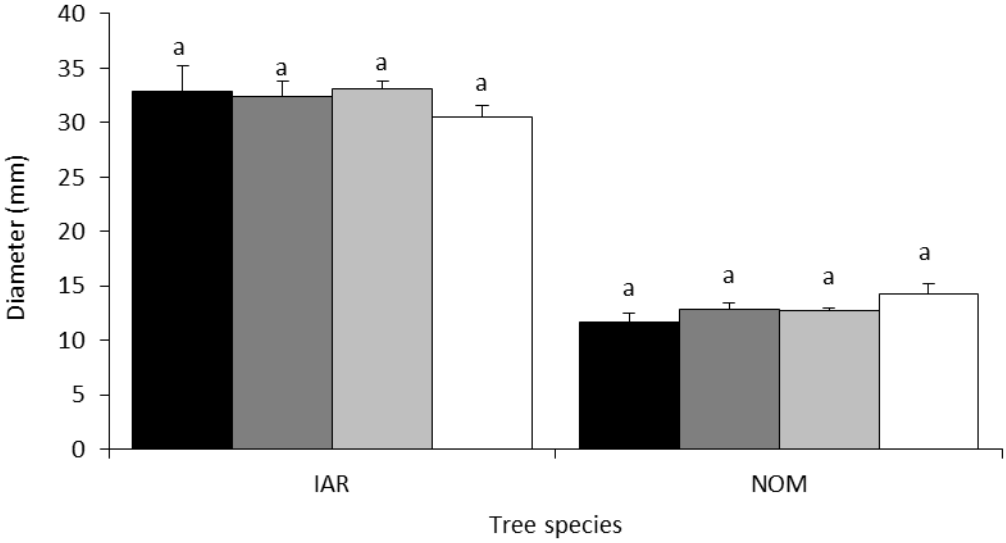

Figure 5. Mean (± SE) diameter (mm) of Alnus cordata (IAR) and Acer platanoides (NOM) after 12 months under experimental treatments: Control (■), Earthworm only (▩), composted green waste (CGW) only (▢), CGW plus earthworm (□). Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5.

Supplementary material

Table I. Mean (± SE) effects of experimental treatments on soil chemical parameters after 12 months in mesocosm tubes containing *Alnus cordata* (n=5). Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05 **, p <0.01, *** p <0.001.

			A. cordata			
Soil Type	Chemical parameter	No tree	Control	Earthworm only	CGW only	Earthworm and CGW
0 - 0.2 m bulk Soil	pH	8.52 ± 0.04	8.70 ± 0.08	8.66 ± 0.08	8.48 ± 0.09	8.50 ± 0.03
	Cond. (µs/cm)	1356.98 ± 293.85	1008.68 ± 101.58	1098.1 ± 112.15	1143.1 ± 242.04	867.6 ± 50.83
	Total N (%)	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00ab	0.10 ± 0.00b***	0.10 ± 0.00b***
	C(Org) (%)	1.72 ± 0.03a	1.79 ± 0.07ab	1.94 ± 0.13ab	2.03 ± 0.04ab	2.07 ± 0.10b*
	O.M. (%)	2.96 ± 0.05a	3.08 ± 0.12ab	3.35 ± 0.22ab	3.50 ± 0.07ab	3.56 ± 0.17b*
	C:N ratio	22.54 ± 0.53ab	23.01 ± 0.8ab	23.25 ± 0.78b*	20.59 ± 0.24a	21.30 ± 0.27ab
	moisture content (%)	23.14 ± 0.53b***	16.65 ± 0.98a	16.63 ± 1.34a	14.93 ± 0.69a	16.56 ± 0.450a
	K (mg/kg)	132.27 ± 3.11b**	100.78 ± 6.14a	96.97 ± 3.54a	115.21 ± 3.98ab	113.09 ± 8.74ab
	Ca (mg/kg)	2691.05 ± 113.35	2787.3 ± 58.04	2815.46 ± 82.78	2963.35 ± 76.94	2661.3 ± 43.17
	Mg (mg/kg)	72.36 ± 5.74	61.45 ± 3.94	61.77 ± 5.30	75.97 ± 4.09	73.68 ± 4.17
	Na (mg/kg)	14.86 ± 0.63	14.65 ± 0.38	16.62 ± 0.69	16.73 ± 0.58	15.01 ± 0.54
	[N(NH_4^+)] (mg/kg)	1.13 ± 0.06ab	1.20 ± 0.10b	0.65 ± 0.19a	1.31 ± 0.06b*	1.00 ± 0.153ab
	[N(NO_2^-)] (mg/kg)	0.52 ± 0.33	0.29 ± 0.17	0.10 ± 0.04	0.12 ± 0.02	0.09 ± 0.03
	[N(NO_3^-)] (mg/kg)	0.45 ± 0.06	0.29 ± 0.08	0.21 ± 0.06	0.32 ± 0.04	0.27 ± 0.08
	S(SO_4^{2-}) (mg/kg)	190.33 ± 45.29	124.19 ± 14.95	159.58 ± 25.8	202.1 ± 90.15	86.17 ± 9.46
	P(PO_4^{3-}) (mg/kg)	21.82 ± 1.04b***	18.69 ± 0.7a	18.65 ± 0.37a	22.12 ± 0.53b***	23.48 ± 0.63b***
0 - 0.2 m rhizosphere soil	pH	N/A	8.48 ± 0.11	8.62 ± 0.09	8.40 ± 0.09	8.58 ± 0.07
	Cond. (µs/cm)	N/A	1039.5 ± 146.65	1073.0 ± 146.68	752.1 ± 32.41	881.0 ± 13.68
	Total N (%)	N/A	0.12 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
	C(Org) (%)	N/A	2.72 ± 0.24	2.25 ± 0.10	2.42 ± 0.11	2.58 ± 0.14
	O.M. (%)	N/A	4.68 ± 0.41	3.89 ± 0.17	4.16 ± 0.19	4.46 ± 0.24
	C:N ratio	N/A	23.13 ± 0.48b	22.5 ± 0.44b	19.83 ± 0.44a***	21.91 ± 0.61b
	moisture content (%)	N/A	22.65 ± 1.62	18.20 ± 1.89	17.18 ± 1.26	19.19 ± 1.17
	K (mg/kg)	N/A	91.09 ± 3.9	92.73 ± 3.76	96.9 ± 6.77	100.75 ± 2.75
	Ca (mg/kg)	N/A	3159.25 ± 171.27	2934.24 ± 131.38	2994.6 ± 154.01	3120.36 ± 56.64
	Mg (mg/kg)	N/A	101.73 ± 6.93	91.97 ± 5.96	99.52 ± 6.15	103.16 ± 7.66
	Na (mg/kg)	N/A	21.97 ± 0.96	21.68 ± 1.09	21.79 ± 1.61	21.12 ± 1.46
	[N(NH_4^+)] (mg/kg)	N/A	2.12 ± 0.28b*	1.04 ± 0.14a	1.90 ± 0.35ab	1.22 ± 0.31ab
	[N(NO_2^-)] (mg/kg)	N/A	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.08 ± 0.03
	[N(NO_3^-)] (mg/kg)	N/A	0.23 ± 0.05	0.14 ± 0.01	0.23 ± 0.06	0.22 ± 0.05
	S(SO_4^{2-}) (mg/kg)	N/A	77.84 ± 23.5	85.95 ± 21.65	47.54 ± 8.10	50.76 ± 10.29
	P(PO_4^{3-}) (mg/kg)	N/A	19.48 ± 1.18ab	18.65 ± 0.56a	23.11 ± 1.48b*	22.95 ± 1.52ab
0.2 - 0. 4 m bulk soil	pH	9.08 ± 0.44	8.78 ± 0.34	8.62 ± 0.07	8.44 ± 0.09	8.62 ± 0.07
	Cond. (µs/cm)	827.80 ± 100.41a	1691.6 ± 334.68b*	936.3 ± 127.12ab	877.7 ± 148.49a	841.1 ± 71.56a
	Total N (%)	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.12 ± 0.00b***	0.1 ± 0.00c***
	C(Org) (%)	1.72 ± 0.04a	1.86 ± 0.05a	1.83 ± 0.06a	2.23 ± 0.04b***	2.16 ± 0.07b***
	O.M. (%)	2.97 ± 0.06a	3.21 ± 0.08a	3.15 ± 0.10a	3.84 ± 0.07b***	3.72 ± 0.12b***
	C:N ratio	22.88 ± 1.04b**	23.73 ± 1.02b**	22.54 ± 0.52b**	19.31 ± 0.30a	21.71 ± 0.51ab
	moisture content (%)	27.14 ± 0.84b***	17.01 ± 1.18a	18.19 ± 1.68a	16.06 ± 1.01a	17.97 ± 0.96a
	K (mg/kg)	123.93 ± 2.66b***	87.85 ± 4.08a	83.06 ± 2.89a	96.89 ± 5.82a	99.49 ± 5.13a
	Ca (mg/kg)	2880.70 ± 313.66	3059.31 ± 214.48	2710.74 ± 75.05	2867.89 ± 106.31	2917.29 ± 56.77
	Mg (mg/kg)	66.38 ± 4.97	59.23 ± 4.46	66.01 ± 3.81	71.74 ± 5.41	71.66 ± 4.40
	Na (mg/kg)	14.85 ± 0.49	16.21 ± 0.73	18.11 ± 0.58	17.78 ± 1.35	17.44 ± 0.91
	[N(NH_4^+)] (mg/kg)	1.06 ± 0.05	0.70 ± 0.12	0.40 ± 0.18	0.91 ± 0.20	0.90 ± 0.23
	[N(NO_2^-)] (mg/kg)	0.36 ± 0.23	0.26 ± 0.15	0.05 ± 0.02	0.11 ± 0.01	0.14 ± 0.05
	[N(NO_3^-)] (mg/kg)	0.53 ± 0.06b***	0.18 ± 0.02a	0.16 ± 0.05a	0.22 ± 0.06a	0.29 ± 0.06a
	S(SO_4^{2-}) (mg/kg)	87.49 ± 13.53a	287.07 ± 79.67b*	151.68 ± 54.98ab	91.02 ± 16.81a	95.88 ± 15.39a

Table II. Mean (± SE) effects of experimental treatments on soil chemical parameters after 12 months in mesocosm tubes containing *Acer platanoides* (n=5). Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05 **, p <0.01, *** p <0.001.

			A. platanoides			
Soil Type	Chemical parameter	No tree	Control	Earthworm only	CGW only	Earthworm and CGW
0 - 0.2 m Bulk Soil	pH	8.52 ± 0.04	8.72 ± 0.05	8.62 ± 0.13	8.92 ± 0.11	8.72 ± 0.10
	Cond. (µs/cm)	1356.98 ± 293.85a	761.6 ± 62.52ab	949.7 ± 75.48ab	757.9 ± 57.54ab	684.6 ± 98.89b*
	Total N (%)	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.1 ± 0.00b***	0.09 ± 0.00b***
	C(Org) (%)	1.72 ± 0.03a	1.69 ± 0.02a	1.75 ± 0.02a	2.09 ± 0.05c***	1.93 ± 0.02b***
	O.M. (%)	2.96 ± 0.05a	2.91 ± 0.03a	3.02 ± 0.08a	3.6 ± 0.09b***	3.33 ± 0.03c***
	C:N ratio	22.54 ± 0.53ab	22.25 ± 0.55ab	23.38 ± 0.50b*	20.85 ± 0.66a	20.87 ± 0.78a
	moisture content (%)	23.14 ± 0.58	21.24 ± 0.71	20.69 ± 0.83	19.36 ± 1.78	20.10 ± 0.68
	K (mg/kg)	132.27 ± 3.11a	129.05 ± 2.86ab	125.16 ± 4.68ab	150.65 ± 8.05b*	140.58 ± 7.59ab
	Ca (mg/kg)	2691.05 ± 113.35	2777.75 ± 54.43	2745.83 ± 36.9	2972.11 ± 90.24	2779.5 ± 135.63
	Mg (mg/kg)	72.36 ± 5.74	89.62 ± 8.36	75.82 ± 6.53	87.56 ± 4.55	88.95 ± 4.88
	Na (mg/kg)	14.86 ± 0.63	15.98 ± 0.63	15.93 ± 0.67	16.56 ± 0.64	15.36 ± 0.64
	$[N(NH_4^+)]$ (mg/kg)	1.13 ± 0.06ab	1.31± 0.07ab	0.93 ± 0.20a	1.49 ± 0.11b*	1.41 ± 0.08ab
	$[N(NO_2^-)]$ (mg/kg)	0.52 ± 0.33	0.14 ± 0.03	0.17 ± 0.06	0.12 ± 0.05	0.44 ± 0.16
	$[N(NO_3^-)]$ (mg/kg)	0.45 ± 0.06	0.39 ± 0.05	0.35 ± 0.10	0.58 ± 0.27	0.56 ± 0.09
	$S(SO_4^{2-})$ (mg/kg)	190.33 ± 45.29	103.47 ± 12.68	166.93 ± 55.68	79.8 ± 19.58	92.6 ± 31.03
	$P(PO_4^{3-})$ (mg/kg)	21.82 ± 1.04a	21.41 ± 1.01a	21.14 ± 0.51a	26.86 ± 1.58b**	25.87 ± 1.42ab
	pH	N/A	8.46 ± 0.09	8.52 ± 0.06	8.32 ± 0.09	6.82 ± 1.71
0 - 0.2 m Rhizo soil	Cond. (µs/cm)	N/A	845.7 ± 277.84	565.3 ± 409.5	314.6 ± 193.4	446.9 ± 195.37
	Total N (%)	N/A	0.12 ± 0.01	0.11 ± 0.01	0.15 ± 0.02	0.10 ± 0.03
	C(Org) (%)	N/A	3.35 ± 0.42	3.02 ± 0.45	4.22 ± 0.68	2.35 ± 0.64
	O.M. (%)	N/A	5.77 ± 0.72	5.21 ± 0.78	7.27 ± 1.16	4.05 ± 1.11
	C:N ratio	N/A	27.74 ± 1.73a	27.82 ± 2.31a	28.06 ± 1.13a	17.97 ± 4.54b*
	moisture content (%)	N/A	34.36 ± 2.73	30.93 ± 3.93	29.58 ± 3.30	25.95 ± 2.21
	K (mg/kg)	N/A	114.34 ± 4.32	136.49 ± 5.1	131.43 ± 8.78	111.37 ± 27.92
	Ca (mg/kg)	N/A	3465.21 ± 280.22	3377.16 ± 97.96	3656.88 ± 284.05	2663.15 ± 699.1
	Mg (mg/kg)	N/A	148.54 ± 14.86	147.29 ± 7.56	151.93 ± 20.17	115.13 ± 31.56
	Na (mg/kg)	N/A	21.88 ± 1.83	23.96 ± 2.11	22.59 ± 0.80	17.64 ± 4.68
	$[N(NH_4^+)]$ (mg/kg)	N/A	2.30 ± 0.44	1.64 ± 0.23	3.01 ± 0.53	2.37 ± 0.21
	$[N(NO_2^-)]$ (mg/kg)	N/A	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.01	0.11 ± 0.04
	$[N(NO_3^-)]$ (mg/kg)	N/A	0.38 ± 0.08	0.61 ± 0.09	0.43 ± 0.20	0.47 ± 0.07
	$S(SO_4^{2-})$ (mg/kg)	N/A	99.81 ± 47.15	114.86 ± 47.69	36.33 ± 5.05	31.3 ± 9.14
	$P(PO_4^{3-})$ (mg/kg)	N/A	22.35 ± 1.41	26.13 ± 1.81	25.12 ± 2.27	23.3 ± 5.93
0.2 - 0.4 m Bulk soil	pH	9.08 ± 0.44	8.9 ± 0.08	8.62 ± 0.09	8.68 ± 0.14	8.50 ± 0.06
	Cond. (µs/cm)	827.8 ± 100.41ab	1186.5 ± 171.09b**	757.6 ± 86.12a	694.8 ± 17.1a	595 ± 33.41a
	Total N (%)	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.10 ± 0.01b***	0.11 ± 0.00b***
	C(Org) (%)	1.72 ± 0.04	1.79 ± 0.04	1.81 ± 0.05	2.10 ± 0.05	2.16 ± 0.07
	O.M. (%)	2.97 ± 0.06a	3.08 ± 0.06a	3.13 ± 0.09a	3.62 ± 0.08b***	3.73 ± 0.12b***
	C:N ratio	22.88 ± 1.04ab	23.19 ± 1.13ab	24.15 ± 1.28b**	20.31 ± 0.59ab	19.43 ± 0.52a
	moisture content (%)	27.14 ± 0.84a	22.55 ± 1.46	24.01 ± 0.41	21.02 ± 2.01	22.80 ± 0.52
	K (mg/kg)	123.93 ± 2.66	111.05 ± 4.36	117.62 ± 5.93	128.33 ± 5.77	125.26 ± 4.60
	Ca (mg/kg)	2880.7 ± 313.66	2939.5 ± 203.53	2643.66 ± 107.06	2958.92 ± 194.68	2718.28 ± 83.27
	Mg (mg/kg)	66.38 ± 4.97a	66.66 ± 5.27a	77.71 ± 4.71ab	78.06 ± 6.38ab	90.82 ± 3.32b*
	Na (mg/kg)	14.85 ± 0.49	15.34 ± 0.83	16.21 ± 0.75	17.08 ± 1.20	17.27 ± 0.66
	$[N(NH_4^+)]$ (mg/kg)	1.06 ± 0.05ab	1.03 ± 0.08	0.75 ± 0.13	1.43 ± 0.16	1.21 ± 0.10
	$[N(NO_2^-)]$ (mg/kg)	0.36 ± 0.23	0.36 ± 0.35	0.58 ± 0.08	0.13 ± 0.05	0.15 ± 0.04
	$[N(NO_3^-)]$ (mg/kg)	0.53 ± 0.06	0.53 ± 0.063	0.78 ± 0.35	0.29 ± 0.13	0.37 ± 0.30
	$S(SO_4^{2-})$ (mg/kg)	87.49 ± 13.53ab	209.08 ± 57.97b*	99.66 ± 27.74ab	110.52 ± 32.61ab	48.42 ± 8.02a
	$P(PO_4^{3-})$ (mg/kg)	20.38 ± 0.59a	21.11 ± 0.34a	20.55 ± 0.49a	24.64 ± 1.86b*	24.93 ± 1.33b*

Table III. Mean (± SE) chemical content (%) of tree sections of *Alnus cordata* and *Acer platanoides* after 12 months in tubes containing different experimental treatments. Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05 **, p <0.01, *** p <0.001.

Tree section	Element	*A. cordata*				*A. platanoides*			
		Control	EW only	CGW only	EW and CGW	Control	EW only	CGW only	EW and CGW
Branch	N	1.04 ± 0.06	0.96 ± 0.06	1.08 ± 0.07	0.98 ± 0.04	0.43 ± 0.03	0.44 ± 0.02	0.45 ± 0.02	0.46 ± 0.02
	C	50.64 ± 0.23	50.68 ± 0.24	50.94 ± 0.20	50.38 ± 0.22	48.33 ± 0.22	48.15 ± 0.32	47.78 ± 0.25	48.24 ± 0.25
	K	0.57 ± 0.02	0.56 ± 0.04	0.61 ± 0.02	0.60 ± 0.01	0.76 ± 0.07	0.81 ± 0.08	0.90 ± 0.09	0.84 ± 0.05
	Ca	0.76 ± 0.05	0.67 ± 0.04	0.69 ± 0.05	0.64 ± 0.06	1.20 ± 0.15	1.15 ± 0.07	1.16 ± 0.07	1.08 ± 0.08
	Mg	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.00	0.11 ± 0.01
	P	0.07 ± 0.00	0.08 ± 0.01	0.08 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Stem	N	0.46 ± 0.03	0.53 ± 0.01	0.47 ± 0.02	0.51 ± 0.03	0.28 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.28 ± 0.02
	C	49.36 ± 0.01a	50.23 ± 0.12b***	49.87 ± 0.07ab	50.24 ± 0.22b***	48.63 ± 0.16	48.92 ± 0.28	48.76 ± 0.17	48.69 ± 0.16
	K	0.28 ± 0.01	0.30 ± 0.01	0.28 ± 0.02	0.32 ± 0.02	0.35 ± 0.02	0.36 ± 0.03	0.40 ± 0.01	0.40 ± 0.03
	Ca	0.29 ± 0.06	0.41 ± 0.04	0.35 ± 0.03	0.39 ± 0.04	0.36 ± 0.03	0.36 ± 0.01	0.38 ± 0.04	0.34 ± 0.04
	Mg	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
	P	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Leaves	N	2.83 ± 0.06	2.88 ± 0.15	2.91 ± 0.07	2.81 ± 0.06	1.20 ± 0.05	1.21 ± 0.07	1.23 ± 0.05	1.31 ± 0.06
	C	53.13 ± 0.22	52.89 ± 0.20	53.01 ± 0.09	52.9 ± 0.13	48.98 ± 0.31	48.66 ± 0.23	49.22 ± 0.30	49.11 ± 0.21
	K	0.66 ± 0.02	0.74 ± 0.06	0.73 ± 0.04	0.70 ± 0.01	0.86 ± 0.03	0.96 ± 0.09	1.03 ± 0.10	0.99 ± 0.07
	Ca	0.94 ± 0.07	0.94 ± 0.07	0.87 ± 0.07	0.99 ± 0.10	1.34 ± 0.04	1.32 ± 0.08	1.05 ± 0.09	1.16 ± 0.10
	Mg	0.13 ± 0.01	0.14 ± 0.00	0.14 ± 0.01	0.13 ± 0.01	0.20 ± 0.01	0.20 ± 0.02	0.17 ± 0.01	0.18 ± 0.02
	P	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
Fine root 0-0.2 m	N	1.39 ± 0.10	1.22 ± 0.04	1.16 ± 0.12	1.39 ± 0.14	0.73 ± 0.02	0.65 ± 0.07	0.73 ± 0.06	0.84 ± 0.18
	C	44.62 ± 1.51	44.80 ± 0.89	44.73 ± 0.62	45.93 ± 0.62	45.43 ± 0.78	44.34 ± 1.96	43.35 ± 2.85	37.43 ± 3.71
	K	0.43 ± 0.02	0.44 ± 0.02	0.47 ± 0.03	0.45 ± 0.01	0.76 ± 0.03	0.79 ± 0.03	0.77 ± 0.05	0.82 ± 0.06
	Ca	1.45 ± 0.08ab	1.69 ± 0.06b*	1.41 ± 0.08a	1.42 ± 0.05ab	1.64 ± 0.19	1.53 ± 0.08	1.50 ± 0.09	1.88 ± 0.35
	Mg	0.17 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.29 ± 0.07	0.21 ± 0.03	0.21 ± 0.02	0.25 ± 0.01
	P	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.15 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.15 ± 0.02
Fine root 0.2-0.4 m	N	0.94 ± 0.04	1.01 ± 0.04	0.95 ± 0.04	1.03 ± 0.03	0.60 ± 0.03	0.52 ± 0.04	0.54 ± 0.03	0.53 ± 0.04
	C	44.49 ± 1.26	44.47 ± 1.25	43.06 ± 1.56	46.11 ± 0.47	34.6 ± 1.79	34.83 ± 2.73	29.61 ± 1.89	30.48 ± 1.87
	K	0.44 ± 0.02	0.47 ± 0.02	0.45 ± 0.03	0.51 ± 0.02	0.89 ± 0.05	0.87 ± 0.07	0.84 ± 0.06	0.81 ± 0.04
	Ca	1.65 ± 0.14	1.73 ± 0.15	1.65 ± 0.09	1.54 ± 0.10	1.88 ± 0.12	1.81 ± 0.14	1.84 ± 0.08	1.97 ± 0.16
	Mg	0.17 ± 0.02	0.18 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.27 ± 0.02	0.23 ± 0.01	0.27 ± 0.02	0.29 ± 0.02
	P	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.08 ± 0.00	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.02	0.15 ± 0.02
Main root	N	0.7 ± 0.05	0.80 ± 0.10	0.71 ± 0.05	0.70 ± 0.03	0.35 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.37 ± 0.01
	C	48.42 ± 0.16	48.45 ± 0.47	48.05 ± 0.52	48.61 ± 0.24	47.53 ± 0.13	47.16 ± 0.23	47.34 ± 0.07	47.63 ± 0.35
	K	0.39 ± 0.03	0.41 ± 0.02	0.42 ± 0.02	0.39 ± 0.00	0.49 ± 0.02	0.52 ± 0.04	0.47 ± 0.02	0.54 ± 0.03
	Ca	0.71 ± 0.11	0.80 ± 0.15	0.69 ± 0.08	0.64 ± 0.06	0.39 ± 0.04	0.46 ± 0.05	0.39 ± 0.03	0.40 ± 0.03
	Mg	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.07 ± 0.00
	P	0.05 ± 0.00	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.00	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.08 0.00

Table IV. Mean (± SE) *Alnus cordata* and *Acer platanoides* above and below-ground biomass (g) after 12 months in different experimental treatments.

	A. cordata				*A. platanoides*			
Tree section	Control	Earthworm only	CGW only	Earthworm and CGW	Control	Earthworm only	CGW only	Earthworm and CGW
Branch	97.36 ± 9.03	75.24 ± 9.56	75.78 ± 6.89	69.25 ± 6.86	2.59 ± 0.85	2.22 ± 0.22	1.61 ± 0.29	4.08 ± 1.12
Leaves	N/A	N/A	N/A	N/A	6.01 ± 1.18	6.43 ± 0.83	6.32 ± 0.90	9.07 ± 2.18
Stem	112.23 ± 9.76	121.21 ± 7.94	138.9 ± 9.95	109.87 ± 6.36	7.84 ± 1.12	10.17 ± 1.60	10.12 ± 1.18	12.56 ± 2.02
Total above	209.59 ± 17.32	196.45 ± 15.39	214.68 ± 14.28	179.12 ± 11.39	16.44 ± 3.10	18.82 ± 2.34	18.05 ± 2.25	25.72 ± 4.91
Fine root 0.2	8.26 ± 1.13	12.48 ± 2.66	13.03 ± 1.65	8.04 ± 0.34	4.08 ± 1.23	3.91 ± 1.11	4.08 ± 0.58	4.03 ± 0.83
Fine root 0.4	17.30 ± 2.78	17.60 ± 3.13	15.76 ± 2.44	14.55 ± 2.26	4.70 ± 1.41	5.07 ± 1.01	5.20 ± 0.84	5.40 ± 0.71
Main root	68.37 ± 9.61	60.90 ± 5.84	74.56 ± 6.92	57.82 ± 6.97	9.03 ± 1.61	9.65 ± 1.23	10.06 ± 1.43	15.06 ± 4.30
Total below	93.92 ± 11.67	90.98 ± 9.11	103.36 ± 8.04	80.41 ± 7.74	17.82 ± 4.13	18.63 ± 2.53	19.34 ± 2.19	24.49 ± 4.99
Total tree	303.51 ± 28.44	287.43 ± 24.50	318.03 ± 18.49	259.53 ± 13.66	34.25 ± 7.17	37.45 ± 4.84	37.39 ± 4.34	50.21 ± 9.78

Table V. ANOVA table for F-value of the effect of CGW addition on reclaimed soil chemical parameters prior to use in field-based PVC mesocosms.

Source of variation	df	Chemical parameter								
		pH	Total N (%)	C (Org) (%)	O.M. (%)	C:N ratio	Ca (mg/kg)	[$N(NO_3^-)$] (mg/kg)	$S(SO_4^{2-})$ (mg/kg)	$P(PO_4^{3-})$ (mg/kg)
		F	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*
Treatment	1	6.05*	52.63***	34.45***	34.45***	47.16***	7.30*	73.38***	59.07***	188.45***

* Significant at p < 0.05. ** Significant at p < 0.01. *** Significant at p < 0.001. n=2.

Table VI. ANOVA table for F-value of the effect of Italian alder on reclaimed soil chemical parameters after 12 months in field-based PVC mesocosms.

Source of variation	df	Chemical parameter					
		C (Org) (%)	O.M. (%)	K	Mg	Moisture	$P(PO_4^{3-})$ (mg/kg)
		F	*F*	*F*	*F*	*F*	*F*
Tree species	1	5.65*	5.65*	54.87***	1.15***	49.28***	6.41***

* Significant at p < 0.05. ** Significant at p < 0.01. *** Significant at p < 0.001. n=2.

Table VII. ANOVA table for F-value of the effect of CGW and earthworm addition on reclaimed soil chemical parameters under Italian alder trees after 12 months in field-based PVC mesocosms.

Source of variation	df	Soil	Chemical parameter					
			Total N (%)	C (Org) (%)	O.M. (%)	C:N ratio	K	$[N(NO_3^-)]$ (mg/kg)
			F	*F*	*F*	*F*	*F*	*F*
Treatment	4	0 - 0.2 m	8.07***	3.45*	3.45*	3.96*	6.35***	1.35***
		0.2 - 0.4 m	41.69***	13.25***	13.25***	-	-	-

* Significant at p < 0.05. ** Significant at p < 0.01. *** Significant at p < 0.001. n=5

Table VIII. ANOVA table for F-value of the effect of CGW and earthworm addition on reclaimed soil chemical parameters under Norway maple trees after 12 months in field-based PVC mesocosms.

Source of variation	df	Soil	Chemical parameter				
			Total N (%)	C (Org) (%)	O.M. (%)	K	$P(PO_4^{3-})$ (mg/kg)
			F	*F*	*F*	*F*	*F*
Treatment	4	0 - 0.2 m	15.32***	24.6***	24.6***	3.19*	5.39*
		0.2 - 0.4 m	23.12*	-	17*	-	4.33*

* Significant at p < 0.05. ** Significant at p < 0.01. *** Significant at p < 0.001. n=5.